Molecular and physiological analysis of drought response in the Mediterranean conifer *Pinus pinaster* Ait.

Nuria de María , María Ángeles Guevara , José Antonio Cabezas , Enrique Sáez-Laguna , Marina de Miguel , Luis Manuel Díaz , Alberto Pizarro , Carmen Collada , David Sánchez-Gómez , Estrella Cadahía , Ismael Aranda , M. Teresa Cervera

Key words: drought response, molecular dissection, functional characterization, Pinus pinaster

Background

Drought is a major environmental stress factor that determines the growth, development and survival of plants living in water scarce environments. Climate change predictions point at increasing dryness over the Mediterranean region. *Pinus pinaster* Ait. is an important Mediterranean conifer subjected to recurrent drought periods. Notwithstanding its relatively small geographical range this species, which is found along a rainfall cline, is characterized by a significant genetic and adaptive diversity. Different morphological and physiological responses appear to play an important role in drought adaptation of this conifer [1]. These complex responses to drought, from perception to transcriptional, metabolic and physiological changes, need to be considered at a global systems biology level to study the multiple interactive components [2]. Integration and analysis of multidisciplinary datasets will likely increase our understanding of molecular mechanisms controlling *Pinus pinaster* response to drought [1].

Methods

Different approaches were designed to analyze the processes involved in *Pinus pinaster* response to drought, all of them based on a progeny from an *ad-hoc* designed full-sib cross (Gal1056xOria6), that segregates for the response to this environmental factor:

Transcriptome and miRNA characterization: cDNA and miRNA libraries were constructed using RNAs extracted from different tissues from Gal1056xOria6 individuals vegetatively propagated and subjected to different treatments. cDNA and miRNAs libraries were sequenced using GS FLX Titanium and Illumina, respectively (3, Sáez-Laguna & Cervera, unpublished results, de María et al, unpublished results). Assembled reads were annotated and differentially expressed genes selected as potential candidates for qRT-PCR studies. SNPs were identified in the transcriptome.

Construction of genetic maps: Genetic maps of different INIA's progenies were developed mainly based on SNP segregation using Illumina SNP arrays (i.e. Infinium, Golden Gate; 3, 4). Additionally, in the frame of the ProCoGen project, an exome capture system is being developed to study segregation on a set of thousands sequences in different conifers and perform conifer comparative mapping.

QTL analysis: Identification of genome regions involved in the genetic control of *Pinus pinaster* response to water stress was carried out searching for associations between morpho-functional and molecular variants in the corresponding linkage maps [3]. This analysis allowed identification of positional candidate genes.

Study of cytosine methylation: Cytosine methylation during drought response was study at different scales: total cytosine methylation (HPLC analysis), genome-wide methylation changes at anonymous CCGG motives (MSAPs; 5) as well as detailed methylation pattern of a collection of candidate genes in response to the stress (collaboration with Dr. Díaz-Sala, UAH).

Global metabolomic analysis: A set of samples were also used to conduct global metabolomic profiling in order to analyze changes associated with drought stress

in different tissues as well as to analyze metabolite composition associated to the response to combined stresses.

Phenotypic analysis: Growth and different morpho-functional traits related to water and carbon plant economy were analyzed for contrasted genotypes in response to the interaction of water stress and different concentrations of atmospheric CO_2. (6, Sanchez-Gómez & Aranda unpublished results).

Results and Conclusions

More than 20 cDNA libraries were constructed representing different tissues and growing conditions. Differential expression analysis is ongoing to identify potential candidate genes that are further subjected to qRT-PCR. Additionally, a 1,536 SNP array has been developed based on SNPs associated to the reference mapping population and used to construct, together with additional SNPs, dense genetic maps [3]. These maps will be further saturated using an exome capture system designed by ProCoGen consortia in collaboration with Dr. Kirst (UFL, USA) for conifer comparative mapping.

Clones from Gal1056xOria6 showed high variability in their response to drought [6], i.e osmotic adjustment capacity differing between clones. Heritability values for stomatal conductance and intrinsic water use efficiency were moderate.

Genetic maps have also been used for the dissection of leaf gas exchange (photosynthesis and stomatal conductance to water vapour), chlorophyll fluorescence parameters and water use efficiency in response to drought, detecting QTLs that explained 10-20% of the observed phenotypic variability for each trait. Untargeted analysis of metabolic profiles allowed the discrimination of genotypes with contrasting drought response. Different metabolites, such as glutamate family amino acids, polyols and lipids, were correlated with some of the ecophysiological traits responding to drought.

Competing interests

The author declares that they have no competing interests.

Aknowledgements

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Drought is a major environmental stress factor that determines the growth, development and survival of plants living in water scarce environments. *Pinus pinaster* Ait. is an important Mediterranean conifer subjected to recurrent drought periods, which will be increased according to climate change predictions (IPCC, 2014). *P. pinaster* shows a significant genetic and adaptive diversity despite its reduced geographical range of distribution. Different morphological and physiological responses appear to play an important role in drought adaptation of this conifer. A better understanding of these complex responses to drought requires a multidisciplinary approach for data generation, analysis and integration, which is partially described in this work:

Materials and experimental design

Elite clone **Gal1056**
+ wood production
- drought response
- cold response

Oria 6
- wood production
+ drought response
+ cold response

162 F_1 segregating progeny

Vegetative propagation

≠ drought intensity and duration

≠ interactions between drought and other factors (i.e a[CO_2])

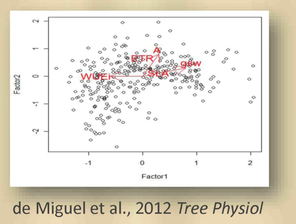
Destructive assays

Non-destructive field assay

Morphological and ecophysiological characterization

Phenotypic characterization
Accurate phenotypic estimates based **on replicated genotypes**

High variability in Gal1056 x Oria6 F_1 full-sib family



de Miguel et al., 2012 *Tree Physiol*

Traits:
Photosynthesis (A_n)
Stomatal conductance (g_{sw})
Chlorophyll fluorescence
$WUE_i = A_n / g_{sw}$
$\delta C13$
SLA = leaf area / leaf biomass
Biomass

Transcriptomics

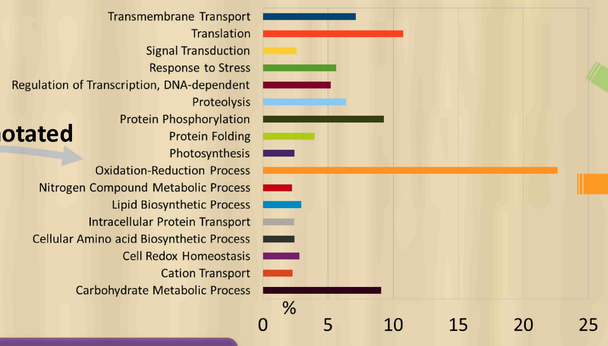
cDNA Libraries
Different tissues (**roots, stems, needles**)
Different treatments (**drought, hormones, control**)

454 GS FLX sequencing

assembly

22,472 isotigs
16,335 isogroups

Functional Annotation

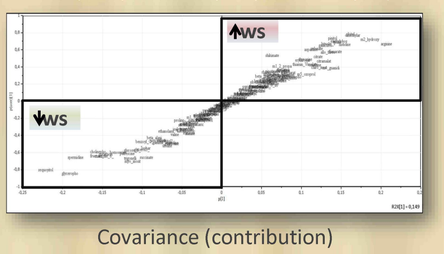


70% annotated

Multi-data integration

Metabolomics

LC-MS (+ESI) + LC-MS (-ESI) + GC-MS (+EI)



Metabolite profiling of Gal1056 X Oria6 genotypes with contrasting drought stress response

Clonal variability in the metabolic response

159 metabolites identified in adult needles from 4 genotypes

53 showed clonal variability

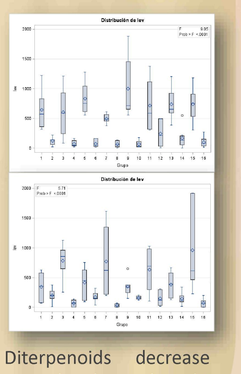
56 were affected by drought

31 showed significant clone x drought interaction

Variability in the metabolic response

Various plant organs to water stress

Adult & young needles to water stress x a[CO_2]



Diterpenoids decrease caused by drought differs between juvenile and mature needles

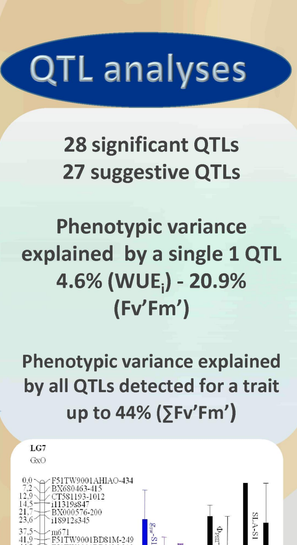
Genetic mapping and trait dissection

QTL analyses

28 significant QTLs
27 suggestive QTLs

Phenotypic variance explained by a single 1 QTL 4.6% (WUE_i) - 20.9% (Fv'Fm')

Phenotypic variance explained by all QTLs detected for a trait up to 44% (∑Fv'Fm')


GxO
LG7
GxO
SLA-SI
SLA-SI
SLA-SI
SLA-SI
SLA-SI
PFAR
PFAR
PFAR